Transferrin receptors in human tissues: their distribution and possible clinical relevance

KEVIN C GATTER*, GEOFFREY BROWN†, IAN S TROWBRIDGE‡, RITA-ELIZABETH WOOLSTON*, DAVID Y MASON*

*From the *Nuffield Department of Pathology, University of Oxford, John Radcliffe Hospital, Oxford, the †Department of Immunology, University of Birmingham Medical School, Edgbaston, Birmingham, and the ‡Department of Cancer Biology, the Salk Institute for Biological Studies, Post Office Box 85300, San Diego, California, USA*

SUMMARY The distribution of transferrin receptors (TR) has been studied in a range of normal and malignant tissues using four monoclonal antibodies, BK19.9, B3/25, T56/14 and T58/1. In normal tissues TR was found in a limited number of sites, notably basal epidermis, the endocrine pancreas, hepatocytes, Kupffer cells, testis and pituitary. This restricted pattern of distribution may be relevant to the characteristic pattern of iron deposition in primary haemachromatosis. In contrast to this limited pattern of expression in normal tissue, the receptor was widely distributed in carcinomas, sarcomas and in samples from cases of Hodgkin's disease. This malignancy-associated expression of the receptor may play a role in the anaemia of advanced malignancy by competing with the bone marrow for serum iron.

The majority of human cells absorb serum iron during their development. This requirement for iron varies considerably; in cells such as developing marrow normoblasts and placental syncytiotrophoblasts iron is required in large amounts for incorporation into haemoglobin or for transport to the developing fetus. In other cells iron is needed in smaller amounts but is nevertheless essential to normal development.

The first step in cellular iron uptake is binding of transferrin:iron complexes to the cell surface membrane. This appears to be mediated by a specific molecule known as the transferrin receptor (TR). There has recently been considerable interest in this molecule which appears to be expressed in much larger amounts on proliferating cells (compared to cells in the resting state) and on cells which have undergone malignant transformation.[1-4] Furthermore TR is similar in several respects to the malignancy-associated glycoprotein reported by Bramwell and Harris.[5] The latter molecule is expressed in substantially greater amounts on malignant than on non-malignant cells and is similar to TR in its molecular weight, its disulphide-bonded dimeric structure, its acidic nature, and its pattern of binding to lectins (concavalin A and wheat germ agglutinin).[3]

Despite these interesting characteristics of the human transferrin receptor there have been no reports of its pattern of tissue distribution in normal and malignant tissue. Previous studies have been largely concerned with TR expression on in vitro human cell lines (Table 1). In the present study we have used four monoclonal antibodies specific for TR to visualise directly the molecule in sections of human tissue. This has revealed a striking distribution pattern in normal tissues, with selective expression in those sites which are most susceptible to excessive iron deposition in primary haemachromatosis—for example, skin, testis, pancreas, liver. Furthermore in a variety of different types of human malignancy it is possible to demonstrate strong immunohistological labelling for TR. This latter observation may be relevant to the development of low serum transferrin concentrations and unexplained anaemia in cases of advanced malignant disease.

Material and methods

PREPARATION OF TISSUE SAMPLES
Fresh samples of normal and malignant tissues were obtained from surgical operation specimens. Samples of placental tissue were obtained immediately after delivery. All samples were immediately frozen in liquid nitrogen and stored in a liquid nitrogen

Table 1 *Expression of transferrin receptor on human cells*

Author(s)	Method of Detection	Results
Larrick, Gresswell (1979)[6]	Binding of ^{125}I-labelled transferrin to human cell lines	TR present on human B and T lymphoblastoid cell lines
Seligman, Schleicher, Allen (1979)[7] } Wada, Hass, Sussman (1979)[8] }	Binding of ^{125}I-labelled transferrin on placental membranes	TR present on human placental brush border membrane (syncytiotrophoblast)
Faulk, Hsi, Stevens (1980)[9]	Immunofluorescence of tissue sections using polyclonal rabbit anti-transferrin antibody	Normal breast negative (100%) Carcinoma of breast positive (72%)
Enns, Shindelman, Tonik, Sussman (1981)[10]	Binding of ^{125}I-labelled transferrin to human cell lines	TR present on placental syncytiotrophoblast, on choriocarcinoma cell line but absent from normal breast cells
Goding, Burns (1981)[11]	Precipitation of TR with monoclonal OKT9—detection by two dimensional electrophoresis	TR present on human T cell leukaemia line CCRF-CEM
Sutherland et al. (1981)[3]	Binding of monoclonal antibody OKT9 with a variety of human cell lines; assessment by ^{125}I-labelled anti-mouse Ig binding or by indirect immunofluorescence	TR present on numerous human leukaemia cell lines and on several carcinoma cell lines (pancreas, melanoma, bladder, rectum, oat cell, teratoma)
Shindelman, Ortmeyer, Sussman (1981)[12]	Binding of ^{125}I-labelled transferrin to human breast tumour cells in suspension	TR present in human breast carcinoma (10 cases) and breast sarcoma (1 case)
Trowbridge, Omary (1981)[2]	Purification from deoxycholate-solubilized membranes by affinity chromatography on a monoclonal antibody-Sepharose column	TR present on cultured human T leukaemia cell lines (RPMI 8402, CCRF-CEM, HPB-ALL)
Lebman et al. (1982)[13]	Immunfluorescence of human cell lines using monoclonal antibody L5.1	Human erythroid precursors, PHA stimulated peripheral blood lymphocytes, colorectal cancer and melanoma cell lines positive. Mature blood cells, most leukaemias and other epithelial cell lines all negative.

Table 2 *Immunoperoxidase labelling of normal tissues with BK19.9. Monoclonal antibodies B3/25, T56/14 and T58/1 were studied on a selection of these tissues**

Tissue	Staining pattern
Skin* Tonsil* Tongue Oesophagus Cervix	Basal layer of epidermis (Fig. 1a and b)
Kidney*	All tubular epithelium (Fig. 1c)
Placenta*	Syncytiotrophoblast (Fig. 1d)
Pancreas*	Islets of Langerhans (Fig. 1e)
Testis*	Seminiferous tubules (Fig. 1f)
Anterior pituitary*	Many positive cells evenly scattered through tissue
Stomach	Mucosal epithelium
Breast	Occasional scattered duct epithelial cells
Liver*	Kupffer cells (Fig. 2a—BK19.9 only) Hepatocytes (Fig. 2b—B3/25, T56, T58)

Tissues showing consistent negative staining: prostate, ovary, thyroid, lung, adrenal cortex, submandibular gland, synovium and colon. In lymphoid tissue scattered weakly stained cells were widely distributed with germinal centres showing diffuse weak labelling of variable intensity. In connective and inflammatory tissue scattered weakly stained cells, often with the morphological appearance of macrophages (including giant cells) were seen.
*Tissues tested with B3/25, T56/14, T58/1.4.

refrigerator. Cryostat sections (5–10 μm in thickness) were collected on gelatin-coated glass slides, thoroughly dried (by desiccation for 4–18 h in the vacuum chamber of a freeze drying apparatus) and fixed in acetone for 10 min at room temperature. Sections were then air-dried and either stained immediately by the immunoperoxidase procedure (see below) or else wrapped in aluminium foil and stored at −20°C.

ANTIBODIES

The production of the monoclonal antibody BK19.9, and the evidence for its specificity for

human TR has been described previously.[14] BK19.9 is of murine origin and was used for immunohistological staining as undiluted tissue culture supernatant. Three other anti-TR monoclonal antibodies, also of murine origin (B3/25, T56/14, T58/1—all from the Salk Institute) of which B3/25 has been extensively characterised[2 15 16] were obtained as Ig purified from ascitic fluid and used at appropriate dilutions in Tris-buffered saline.

A monoclonal antibody reactive with human monocytes (OKM) was purchased from Ortho Pharmaceutical Corporation (Raritan, New Jersey USA).
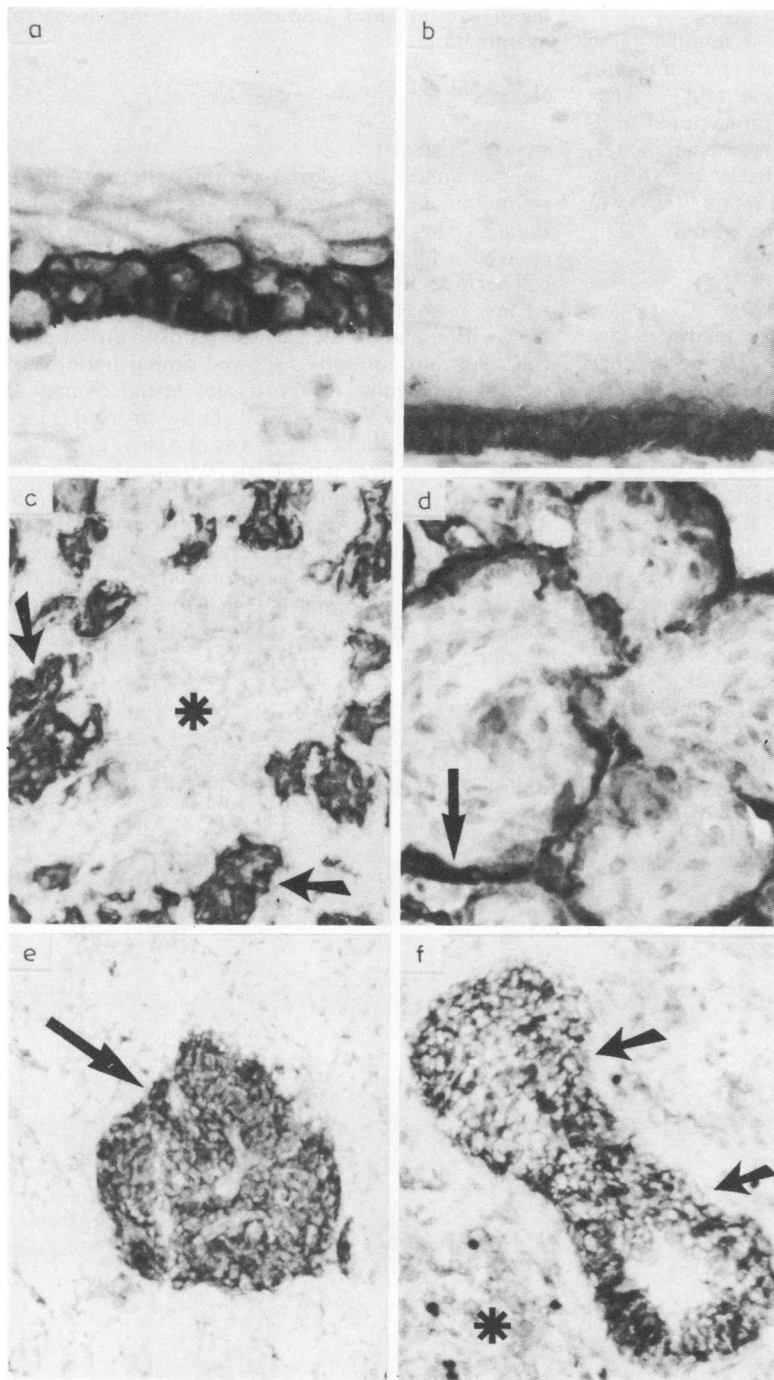
Fig. 1   *Normal tissues labelled by the immunoperoxidase method with the monoclonal anti-transferrin receptor antibody BK19.9. (a) Normal skin showing labelling of the basal layer of the epidermis ×800. (b) Labelling of the basal layer of normal non-keratinising epithelium in the ectocervix ×500. (c) Positively labelled proximal renal tubules (arrow) in kidney contrasting with a negative glomerulus (asterisk) ×200. (d) Positively labelled syncytiotrophoblast (arrow) contrasting with the negative central core of cytotrophoblast in the placenta ×800. (e) Normal pancreas showing positive labelling of an islet of Langerhans (arrow) with surrounding negative exocrine pancreas ×200. (f) Positively labelled testicular tubule (arrows) surrounded by seminoma (*) which is more weakly stained ×200.*

IMMUNOPEROXIDASE REAGENTS

Sheep antiserum against mouse immunoglobulin was raised by a conventional immunisation schedule. Peroxidase:antiperoxidase (PAP) complexes were prepared using the monoclonal anti-peroxidase antibody P6/38.[17] Diaminobenzidine tetrahydrochloride was obtained from the Sigma Chemical Company. Tris-buffered saline (TBS) was prepared by adding a tenth volume of 0·5 M Tris HCl buffer (pH 7·6) to 0·15 M saline.

IMMUNOPEROXIDASE STAINING

Cryostat sections were stained by the three-stage PAP immunoperoxidase procedure. The initial stage involved the application of 50–100 μl of antibody-containing supernatant to dry cryostat sections, followed by incubation for 30 min in a covered chamber. Sections were then washed in TBS and incubated for a further 30 min with sheep antimouse Ig. This reagent was diluted 1/25 in TBS to which normal human serum (at a final dilution of 1/25) had been added in order to block cross-reactivity against human Ig. After a further wash in TBS, monoclonal PAP was applied for 30 min with a final wash in TBS.

Development of the peroxidase reaction was performed by incubating sections with diaminobenzidine (0·6 mg/ml) and hydrogen peroxide (0·01%) in TBS for 5–10 min at room temperature. Sections were then washed in tap water, counterstained with

haematoxylin and mounted for microscopical examination.

Results

NORMAL TISSUES

The immunohistological reaction patterns seen in normal tissues are listed in Table 2 and illustrated in Fig. 1. The majority of positively labelled cells showed a linear reaction product demarcating the cell surface. In addition diffuse cytoplasmic positivity was also often seen suggesting that TR is present both within and on the surface of positively labelled cells. All four antibodies showed similar patterns of reactivity on most of the tissues tested (Table 2) although BK19.9 gave consistently stronger reactions than the others. There was one clear exception in the case of liver where BK19.9 stained Kupffer cells (Fig. 2a) and not hepatocytes whereas B3/25, T56/14 and T58/1.4 gave the reverse pattern (Fig. 2b). The other major difference was that whereas BK19.9 always stained islets of Langerhans in the pancreas extremely clearly (Fig. 1e) the other three consistently gave much weaker and more patchy labelling.

MALIGNANT TISSUES

Antibody BK19.9

A total of 87 neoplastic tissue samples were analysed for the presence of TR by staining with
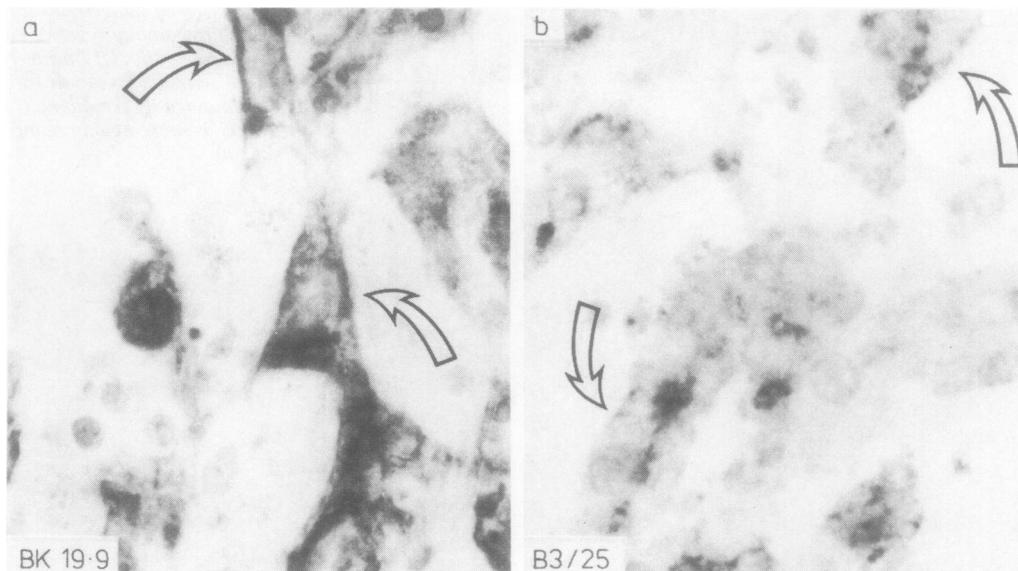

Fig. 2   (a) *Kupffer cells (arrow) in liver, positively labelled by monoclonal antibody BK19.9, contrasting with negative hepatocytes ×800. (b) Hepatocytes (arrow) positively labelled by monoclonal antibody B3/25, in an adjacent section from the same biopsy as illustrated in Fig. 2a ×800.*

Table 3 *Immunoperoxidase labelling of malignant tissue with the anti-transferrin receptor antibody BK19.9*

Adenocarcinoma	No of cases		Squamous carcinoma	No of cases	
	Positive	Negative		Positive	Negative
Breast (ductal)	10	2	Skin	1	—
Breast (lymph node metastasis)	1	—	Lymph node metastasis	1	—
Stomach	1	—	Lung	1	1
Colon	3	—	Cervix	1	—
Colon (carcinoid)	1	—			
Kidney	2	—	*Transitional carcinoma*		
Kidney (lung metastasis)	—	1	Bladder	1	—
Uterus	—	1			
Ovary	1	—	*Sarcoma*		
Ovary (lymph node metastasis)	2	—	Ewing's sarcoma	1	—
Thyroid	—	1	Alveolar soft parts	1	—
Lung	2	—	Fibrosarcoma	2	—
Prostate	—	1			
Prostate (lung metastasis)	—	1	*Germ cell*		
Pancreas (liver metastasis)	2	—	Embryonal carcinoma	1	—
			Seminoma	3	—
			Teratoma (malignant)	1	—
			Lymphoma		
			Hodgkin's	15	—
			Non-Hodgkin's	16	9
			Total	70	17

antibody BK19.9. As seen in Table 3 many of these cases gave positive staining reactions.

Carcinomas Staining was seen in 30 of the 38 cases with most samples showing a strong labelling reaction. The pattern of staining, however, varied; in some cases there was generalised diffuse labelling of all neoplastic cells (Fig. 3), whilst in other cases positively stained cells were present (as isolated scattered cells or in groups) against a background of unstained neoplastic cells.
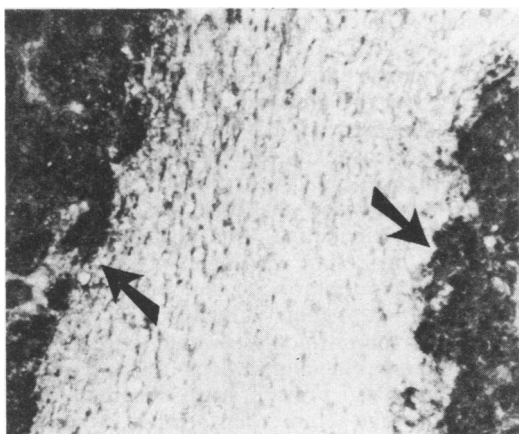

Fig. 3 *Two nodules (arrowed) of secondary adenocarcinoma of the pancreas in liver showing strong positive labelling with the monoclonal antibody BK19.9. The positively labelled scattered cells in the liver (centre) are Kupffer cells ×200.*

Germ cell tumours and sarcomas All nine cases in this group of primitive tumours stained positively for TR.

Non-Hodgkin's lymphoma Among 25 cases, including both nodular and diffuse types of lymphoma, 16 samples showed staining, usually relatively weak, of the neoplastic cell population.

Hodgkin's disease All 15 cases of Hodgkin's disease examined showed weak staining of numerous cells in areas of active disease including obvious mononuclear Hodgkin and multinuclear Reed Sternberg cells. In addition, in cases of nodular sclerosing disease, the inflammatory infiltrate in the fibrous tissue bands contains scattered weakly staining cells, many with the morphological appearance of macrophages. Positive staining with the monoclonal anti-monocyte antibody OKM provided additional evidence for the phagocytic nature of these cells.

Antibodies B3/25, T56/14 and T58/1

A small number of representative neoplastic biopsies were also stained with B3/25, T56/14 and T58/1. All three antibodies gave the same staining pattern, being generally weaker and more patchy than the staining with BK19.9.

Discussion

This study has shown that TR, as detected by four monoclonal antibodies shows a restricted expression pattern in normal human tissue. It is present in the

basal layers of squamous epithelium (both keratinising and non-keratinising—Fig. 1), but not in more superficial cell layers. Furthermore it is found in the endocrine pancreas (but not the exocrine portion of the gland), in seminiferous tubules of the testis, in the pituitary, in Kupffer cells of the liver (Fig. 2a—BK19.9 only) and hepatocytes (Fig. 2b—B3/25, T56/14 and T58/1). Macrophages in many tissues also appear to express TR.

The explanation of this pattern of TR distribution is not immediately apparent. Its selective presence in the basal layers of squamous epithelium is in keeping with reports that it is expressed on rapidly proliferating cells.[23] This may also account for its expression on seminiferous tubules. At other sites, however—for example, endocrine pancreas—such an explanation is not tenable.

Whatever the underlying reasons for these patterns of TR expression it is striking that they show partial correlation with the sites (skin, pancreas, testis, pituitary, and liver) at which excessive iron deposition occurs in primary haemochromatosis. This suggests that tissues which sustain damage in this disease may be susceptible because of high levels of TR expression. It is not possible, however, solely on the basis of this immunohistological analysis, to assess whether this explanation of the pathogenesis of tissue damage in primary haemachromatosis is valid. It should be noted that at least two sites which are not generally recognised as being particularly susceptible to damage from excess iron deposition were clearly labelled with anti-TR (kidney and gut). A striking finding was that hepatocytes and Kupffer cells both of which are known to express TR[18] gave different labelling patterns with BK19.9 compared to the other three monoclonal anti-TR antibodies (Fig. 2). The other major difference between BK19.9 and the other antibodies was that whereas BK19.9 stained islets of Langerhans strongly and completely (Fig. 1e) the others were consistently much weaker and patchier.

The explanation of the fact that four antibodies of apparently identical specificities, gave different immunohistological labelling patterns remains to be elucidated. This discrepancy could reflect the fact that the transferrin receptor is not a single molecular entity but rather a family of molecules which are antigenically similar but not identical. There is a precedent for this in the description[19] of monoclonal antibodies which will distinguish sub-populations of the leucocyte-associated antigen. It is indeed possible that the striking molecular similarity between the transferrin receptor and the malignancy associated antigen[5] (see Introduction) may also be explained by the fact that both belong to a family of structurally related but antigenically distinct molecules concerned with molecular binding and transportation—that is, transferrin and glucose respectively. The recently reported "family" of molecules represented by the macrophage antigen MAC-1 and the lymphoid antigen LFA-1[20 21] provides a precedent in this context.

Finally TR was found to be present on the majority of carcinomas, on all sarcomas and on all cases of Hodgkin's disease examined. In contrast non-Hodgkin's lymphomas were either weakly labelled or gave (in approximately one third of cases) a negative reaction.

These results should be interpreted in the context of previous studies in animals which have shown a relation between the rate of tumour proliferation, tumour mass and the uptake of transferrin by tumours.[22] This suggests that a relation may exist between TR expression and the growth rate or differentiation of human tumours. This is supported by a recent study showing that anti-transferrin receptor antibody inhibited the growth of human melanoma cells in nude mice.[23] However it should be noted that in the present study staining for transferrin receptor was not obviously related to tumour differentiation or mitotic rate, and indeed some secondary tumours were entirely negative for TR (Table 3).

One point of interest that is raised by TR expression in human tumours concerns the pathogenesis of the chronic anaemias which are often associated with advanced malignancy. Such anaemias can only rarely be shown to be due to bone marrow invasion and almost certainly have a multi-factorial aetiology. The major factors causing the anaemia are generally agreed to be increased haemolysis with an inadequate bone marrow erythropoietic response.[24 25] Lamar *et al*[26] have shown in rats with a hepatoma that there is an approximately 50% reduction in iron utilisation by bone marrow, with a concomitant rise in utilisation by the spleen. In view of the striking expression of TR in human malignant cells it seems reasonable that one causative factor in the anaemia associated with cancer could be direct competition between the bone marrow and the tumour for circulating transferrin-bound iron. Although Lamar *et al*[26] were unable to show any net increase in iron utilisation in tumour-bearing rats it is probable that the total amount of iron available for bone marrow normoblasts would decrease as the tumour increases in volume. Such iron utilisation by tumours may also provide an explanation for the observation that serum transferrin is significantly lower in patients with malignant disease.[27]

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Requests for reprints to: Dr KC Gatter, Nuffield Department of Pathology, University of Oxford, John Radcliffe Hospital, Oxford, England.